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Effects of the Preliminary Anatoxin Administration on the Resistance of Mice to Botulinal Toxin

(Preliminary Report)

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Of great theoretical and practical importance is the question of the rate of development of specific resistance to the effects of toxins after administration of anatoxins.

The majority of investigators link the effects of anatoxin with the stimulation of the organism by antigens, in response to which a specific immunity to a toxin develops in 7 to 14 days later. However, KRECH established in 1949 a new fact with the aid of experimental model of tetanic intoxication. He demonstrated that a protective effect of the anatoxin may appear immediately after administration of the latter.

This question has been studied farther on models of the tetanic intoxication by AVERYANOVA (1956), VOROBEV (1958), RAYNAUD et al. (1951), RAYNAUD and WRIGHT (1953), LEMETAYER et al. (1954), GOLDMAN, TURNER and STAFFORD (1954), DAVIES and WRIGHT (1955), etc. It has been confirmed in numerous experiments that the tetanic anatoxin produces a protective effect, which is not combined with the appear-

ance of antitoxin in the blood. Large doses of anatoxin, administered to animals simultaneously, or 12 to 24 hours prior to the injection of 1 or 2 fatal doses of tetanic toxin, saved the animals from death in most cases. The effect of the increased resistance to the toxin appeared at once after administration of the anatoxin and it disappeared several days later.

The effects of the early administration of anatoxin on the development of resistance to the botulinal toxin have not been studied sufficiently. In checking the available to us bibliography, we became familiar with only one investigation, that of KATIČ, who in 1956 published the results of experimental research involving the therapeutic effect of a specific anatoxin on the intoxication caused by botulinal toxin type D. The author was able to notice that a subcutaneous administration of the anatoxin after 24 hours following a subcutaneous injection of the toxin produced a somewhat prolonged duration of life in experimental mice only in such cases, if the animals had been poisoned solely with small doses of the toxin (5 Dlm), but no protective effect has been observed after injection of large doses of the toxin (50 Dlm).

The purpose of the present investigation was to study the effects of the preliminary anatoxin administration on the development of resistance of mice to botulinal toxin. We excluded from this investigation the question of dependency of the protective effect on a dose of anatoxin, also the mechanism of this phenomenon, a possibility of its practical application and other problems connected with this aspect.

The investigative methods were as follows: we diluted a suspension of dry botulinal toxin type A with a normal rabbit serum, calculating 1 mg per 1 ml; the initial dilution of the toxin was stored in a refrigerator until completion of the series of experiments. We determined the strength of toxin by titration on white mice, which weighed 18 to 20 gm each. We prepared 4 practical dilutions of the toxin for each experiment, calculating that the ratio of magnitude of each subsequent experimental dose of the toxin should comprise 1.5 to the previous one. Each dose of the toxin was administered subcutaneously to a group of mice set for a given experiment, and the volume was 0.5 ml of a respective solution.

The harmlessness of the concentrated botulinal anatoxin type A has been checked previously by way of injection to the mice. A subcutaneous, intramuscular and intravenous administration of the anatoxin in 0.25 ml dose had no visible effect on the condition of animals. We used two groups of animals in each experiment; the experimental mice received the anatoxin subcutaneously in 0.25 ml dose at various times, i.e. 24 hours before administration of the toxin, simultaneously, also 24 and 48 hours after the poisoning. The groups of control mice received a proper quantity of the physiological solution. The observations lasted 9 days, because after this time the animals remained alive and no symptoms of sickness have been observed in the survived mice. We recorded the number of dead mice.

The statistical interpretation has been completed according to the modified method of KERBER (ASHMARIN and VORORBV, 1962). We

Table 1

Experiment No.	Dose of toxin (in μg)	Time of administration of anatoxin	Number of animals	Li	G+ G−	LD_{50} and its possible variations (μg)	Reliability of LD_{50} variations according to formula 39
1	25 16.66 11.11 7.4	24 hours before administration of toxin	2/6 0/6 0/6 0/6 2/24	0.33 0 0 0 0.33	≥(+1) ≤(−0.3)	27 (>24 <40)	0.2641 > 0.1027
2	25 16.66 11.11 7.4	Simultaneously with toxin	3/6 2/6 0/6 0/6 5/24	0.5 0.33 0 0 0.83	+0.8 −0.4	22 (19÷30)	0.1761 > 0.112
Control	25 16.66 11.11 7.4		6/6 4/6 1/6 0/6 11/24	1.000 0.666 0.166 0 1.83	+0.5 −0.5	15 (12÷.5)	
3 and 4	25 16.66 11.11 7.4	After 24 hours (experiment 3) and after 48 hours (experiment 4) following administration of toxin	4/5 1/5 0/5 0/5 5/20	0.8 0.2 0 0 1.0	+0.70 −0.50	20 (17÷27)	0.1761 > 0.1310
Control	25 16.66 11.11 7.4		5/5 4/5 1/5 0/5 10/20	1.0 0.8 0.2 0 2.0	+0.55 −0.55	(4 (11÷17)	

Legend: numerator = number of dead animals, denominator = number of animals in experiment; Li = ratio of the number of dead to the total number of animals; G+ G− = intermediate coefficients (determined according to Table IX, manual of I.P. ASHMARIN and A.A. VOROBEV, 1962); 1 μg = $1 \cdot 10^{-9}$ gm.

computed LD_{50} (formula 35), its possible variations (formula 36) and the reliability of various values of LD_{50} in experiments and in control (formula 39). The method of determination of death of 50% of experimental animals, i.e. the LD_{50} rating, was very convenient for the most exact specification of the sensitivity of experimental animals to the toxin. This magnitude increased with the intensified resistance of animals to the toxin, as the dose of toxin, causing death of 50% of animals, enlarged. The method of KERBER enabled us to evaluate, in control and in experiments, the sensitivity of experimental animals to the toxin in a relatively simple way, according to the magnitude of LD_{50} and also to determine the reliability of the increased resistance to the toxin in animals after they received the injection of anatoxin.

The experimental results are presented in Table 1.

The magnitude of LD_{50} of botulinal toxin comprised 14 to 15 mg in mice that received no injection of anatoxin (control groups). Following a preliminary administration of anatoxin to mice 24 hours prior to administration of the toxin (experiment No.1), the value of LD_{50} increased to 25 mg (only 2 mice died out of 24). The increased I_{50} indicated a rise in the resistance of animals to the toxin. Following a simultaneous administration of the anatoxin with the toxin (experiment No.2), the LD_{50} increased from 15 to 22 mg and this also indicated the presence of a definite protective effect of the anatoxin. The same results were obtained in experiments No.3 and 4, in which the anatoxin was administered 24 and 48 hours after the toxin, namely: the lethality was alike in both experiments and

the magnitude of LD_{50} increased from 14 to 20 mg, which also confirmed in this case the presence of the protective effect of administered anatoxin.

We regarded the increased value of LD_{50} after administration of anatoxin as statistically reliable in all 4 experiments. This has been confirmed by computations according to the formula 39. The variations of LD_{50} in experiments and in control are reliable, because the left half of the disparity is greater than the right one (see Table 1, column 8).

The encouraging preliminary data, obtained in the current experiments, may serve as a basis for more detailed studies of the effects of preliminary administration of anatoxin on the development of resistance to botulinal toxin.

Conclusion

Subcutaneously administered to mice 0.25 ml of the concentrated botulinal anatoxin type A, 24 hours before, simultaneously, also 24 and 48 hours after subcutaneous injection of the botulinal toxin type A, increased the value of LD_{50}, which indicated an increased resistance of mice to the toxin, effected by a preliminary administration of the anatoxin to the animals.

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Summary (Engl.)

A study was made of the effect of preliminary toxoid administration on the development of albino mice resistance to botulin. The author presents statistically treated results of 4 experiments on subcutaneous injection of the botulin anatoxin performed 24 hours before, simultaneously with, the subcutaneous injection of botulin, and 24 or 48 hours later. On the basis of the statistically significant changes of LD_{50} under the effect of preliminary administration of toxoid to mice a conclusion was drawn that following the toxoid administratio. the mouse resistance to toxin was rapidly increasing.